

Claims:

1. A method of screening colon tissue for a pathological condition, said method comprising:
measuring Prox-1 expression in a biological sample that comprises
5 colon tissue from a mammalian subject, wherein elevated Prox-1 expression in the colon tissue correlates with a pathological phenotype.
2. A method according to claim 1, comprising comparing Prox-1 expression in the colon tissue to Prox-1 expression in healthy colon tissue, wherein
10 increased Prox-1 expression in the colon tissue from the mammalian subject correlates with a pathological phenotype.
3. A method according to claim 1 or 2, further comprising a step, prior to said measuring step, of obtaining the biological sample comprising colon
15 tissue from a mammalian subject.
4. The method according to any one of claims 1-3, wherein the pathological condition is colon cancer, and wherein increased Prox-1 expression in the colon tissue is indicative of a cancerous or precancerous condition.
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5. The method according to any one of claims 1-4, wherein the measuring comprises measuring Prox-1 protein in the biological sample.
6. The method of claim 5, wherein the measuring comprises
25 contacting the colon tissue with a Prox-1 antibody or antigen-binding fragment thereof.
7. The method of any one of claims 1-6, wherein the measuring comprises measuring Prox-1 mRNA in the colon tissue.

8. The method of claim 7, wherein the measuring comprises *in situ* hybridization to measure Prox-1 mRNA in the colon sample.

5 9. The method of claim 7, wherein the measuring comprises steps of isolating mRNA from the colon tissue and measuring Prox-1 mRNA in the isolated mRNA.

10 10. The method according to any one of claims 1-9, wherein the measuring comprises quantitative polymerase chain reaction (PCR) to quantify Prox-1 mRNA in the colon tissue relative to Prox-1 mRNA in healthy colon tissue.

15 11. A method according to any one of claims 1-10, further comprising measuring expression of at least one gene selected from the group consisting of CD44, Enc1, and ID2 in the colon tissue, wherein elevated Prox-1 expression and elevated expression of the at least one gene in the colon tissue correlate with a pathological phenotype.

20 12. A method according to any one of claims 1-11, further comprising measuring activation of β -catenin/TCF pathway in the colon tissue, wherein activation of the β -catenin/TCF pathway and elevated Prox-1 expression in the colon tissue correlate with a pathological phenotype.

25 13. A method according to claim 12, wherein activation of the β -catenin/TCF pathway is measured by at least one indicator in the colon tissue selected from the group consisting of: mutations in an APC gene; mutations in a β -catenin gene; and nuclear localization of β -catenin.

14. The method according to any one of claims 1-13, wherein the mammalian subject is a human.

15 A method according to claim 14, further comprising a step of administering to a human subject identified as having a pathological condition characterized by increased Prox-1 expression in colon tissue a composition comprising a Prox-1 inhibitor.

16 Use of a molecule that suppresses expression or activity of Prox-1 in the manufacture of a medicament for the treatment of colorectal cancer.

17 A method of inhibiting the growth of colorectal cancer cells in a mammalian subject comprising the step of:

15 administering to the subject a composition comprising a molecule that suppresses expression or activity of Prox-1, thereby inhibiting the growth of colon carcinoma cells.

18 A method or use according to claim 16 or 17, wherein the molecule suppresses Prox-1 expression.

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19 A method or use according to any one of claims 16-18, comprising a step of identifying a mammalian subject with a colon cancer characterized by increased Prox-1 expression, wherein the composition is administered after the identifying step.

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20 A method or use according to any one of claims 16-19, wherein the cancer is selected from a colorectal adenoma and a colorectal carcinoma.

21. The method or use according to any one of claims 16-20, wherein the composition further comprises a pharmaceutically acceptable diluent, adjuvant, or carrier medium.

5 22. The method or use according to any one of claims 16-21, wherein the molecule comprises an antisense oligonucleotide that inhibits Prox-1 expression.

23. The method or use according to any one of claims 16-21,
10 wherein the molecule comprises micro-RNA that inhibits Prox-1 expression.

24. The method or use according to any one of claims 16-21, wherein the molecule comprises short interfering RNA (siRNA) that inhibits Prox-1 expression.

15 25. The method or use of claim 24, wherein the siRNA comprises at least one nucleotide sequence set forth in SEQ ID NOS: 4, 5, 6, and 7.

26. The method or use according to any one of claims 16-21,
20 wherein the molecule comprises a zinc finger protein that inhibits Prox-1 expression.

27. The method or use according to any one of claims 16-21, wherein the molecule comprises a dominant negative form of Prox-1 protein, or an expression vector containing a nucleotide sequence encoding the dominant negative
25 Prox-1 protein.

28. The method or use of claim 27, wherein the dominant negative form of Prox-1 protein has a disrupted DNA binding domain.

29. The method or use of claim 27, wherein the dominant negative form of Prox-1 protein has a disrupted transactivation domain.

5 30. The method or use according to any one of claims 16-21, wherein the molecule comprises short hairpin RNA (shRNA) that inhibits Prox-1 expression.

10 31. The method according to any one of claims 17-30, wherein the composition is administered in an amount effective to suppress Prox-1 expression and increase Notch 1 signaling.

15 32. The use according to any one of claims 16-30, wherein the molecule is present in the composition in an amount effective to suppress Prox-1 expression and increase Notch-1 signaling.

20 33. The method according to any one of claims 17-31, wherein the composition is administered in an amount effective to increase 15-PDGH activity or decrease prostaglandin D2 synthase activity.

34. The method according to any one of claims 17-31, further comprising administering to the subject an inhibitor of the β -catenin/TCF signaling pathway.

25 35. The use according to any one of claims 16-30, wherein the medicament further includes an inhibitor of the β -catenin/TCF signaling pathway.

36. The method or use of claim 34 or 35, wherein the inhibitor of the β -catenin/TCF signaling pathway is dominant negative form of TCF-4.

37. The method or use of claim 34 or 35, wherein the inhibitor of the β -catenin/TCF signaling pathway targets TCF-4, β -catenin, or c-myc.

38. The method according to any one of claims 17-31, further comprising administering to the subject a COX-2 inhibitor.

39. The use according to any one of claims 16-30, wherein the medicament further includes a COX-2 inhibitor.

40. The method or use of claim 38 or 39, wherein the COX-2 inhibitor is a non-steroid anti-inflammatory drug.

41. The method according to any one of claims 17-31, further comprising administering to the subject a Notch signaling pathway agonist.

42. The use according to any one of claims 16-30, wherein the medicament further includes a Notch signaling pathway antagonist.

43. The method or use according to claim 41 or 42, wherein the Notch signaling pathway agonist is a Notch ligand.

44. The method or use of claim 43, wherein the Notch ligand is Jagged1, Jagged2, Delta1, Delta3, Delta4, or Serrate.

45. The method or use of claim 41 or 42, wherein the Notch signaling pathway agonists are Notch targets Hey1, Hey2, or Hes1.

46. A method of inhibiting Prox-1 function in a mammalian subject
5 having a disease characterized by Prox-1 overexpression in cells, comprising the step of administering to said mammalian subject a composition, said composition comprising a compound effective to inhibit Prox-1 function in cells.

47. Use of an inhibitor of Prox-1 function in mammalian cells for
10 the manufacture of a medicament for inhibiting Prox-1 function.

48. A method of screening for a Prox-1 modulator, comprising steps of:

contacting a test molecule with Prox-1 protein, or a nucleic acid
15 comprising a nucleotide sequence that encodes Prox-1 protein, under conditions which permit the interaction of the test molecule with the Prox-1 protein or nucleic acid;

and measuring interaction between the test molecule and Prox-1 protein or nucleic acid, wherein a test molecule that binds the Prox-1 protein or
20 nucleic acid is identified as a Prox-1 modulator.

49. The method of claim 48, wherein the test molecule comprises a protein, a carbohydrate, a lipid, or a nucleic acid.

50. The method of claim 48, wherein the test molecule comprises a
25 member of a chemical library.

51. The method of any one of claims 48-50, comprising measuring the binding between the test molecule and the DNA binding domain of Prox-1.

52. A method of screening for modulators of binding between a DNA and Prox-1 protein comprising steps of:

5 a) contacting a DNA with a Prox-1 protein in the presence and in the absence of a putative modulator compound;

b) detecting binding between the DNA and the Prox-1 protein in the presence and absence of the putative modulator compound; and

10 c) identifying a modulator compound based on a decrease or increase in binding between the DNA and the Prox-1 protein in the presence of the putative modulator compound, as compared to binding in the absence of the putative modulator compound.

53. A method of screening for modulators of binding between a DNA and Prox-1 protein comprising steps of:

15 a) contacting a DNA with a Prox-1 protein in the presence and in the absence of a putative modulator compound;

b) detecting binding between the DNA and the Prox-1 protein in the presence and absence of the putative modulator compound; and

20 c) identifying a modulator compound based on a decrease or increase in differentiation in the presence of the putative modulator compound, as compared to differentiation in the absence of the putative modulator compound.

54. A method according to any one of claims 48-53, further comprising steps of:

25 contacting a cell from a colorectal cancer or colorectal cancer cell line with the Prox-1 modulator; and

selecting a Prox-1 modulator that inhibits growth of the cell.

55. A method according to claim 54, further comprising:
formulating a composition comprising the selected Prox-1 modulator
and a pharmaceutically acceptable carrier;
administering the composition to a mammalian subject having a
5 colorectal cancer; and
monitoring the mammalian subject for growth, metastasis, shrinkage,
or disappearance of the colorectal cancer.

56. A small interfering RNA (siRNA) molecule that comprises a
10 sense region and an antisense region, wherein said antisense region comprises
sequence complementary to a nucleotide sequence encoding Prox-1 set forth as SEQ
ID NO: 2, or a fragment thereof, and wherein the sense region comprises sequence
complementary to the antisense region, or a fragment thereof.

15 57. The siRNA molecule of claim 56, wherein said siRNA
molecule comprises two nucleic acid fragments, wherein one fragment comprises the
sense region and the second fragment comprises the antisense region.

58. The siRNA molecule of claim 57, wherein said sense region
20 comprises a 3'-terminal overhang relative to the antisense region.

59. The siRNA molecule of claim 57 or 58, wherein the antisense
region comprises a 3'-terminal overhang relative to the sense region.

25 60. The siRNA molecule of claim 59, wherein said 3'-terminal
overhangs each comprise 1-5 nucleotides.

61. The siRNA molecule of claim 59, wherein said antisense region
3'-terminal nucleotide overhang is complementary to RNA encoding Prox-1.

62. The siRNA molecule according to any one of claims 56-61, wherein said complementary sequences are 18-100 nucleotides in length.

5 63. The siRNA molecule according to any one of claims 56-61, wherein said complementary sequences are 18-30 nucleotides in length.

64. The siRNA molecule according to any one of claims 56-61, wherein said complementary sequences are 21-23 nucleotides in length.

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65. The siRNA molecule according to any one of claims 56-61, wherein said antisense region comprises sequence complementary to sequence having any of SEQ ID NOs. 4 and 6.

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66. The siRNA molecule according to any one of claims 56-61, wherein said antisense region comprises sequence having any of SEQ ID NOs. 5 and 7.

67. The use of an siRNA molecule according to any one of claims 20 56-66 in the manufacture of a medicament for the treatment of colorectal cancer.

68. The use according to claim 16, wherein the molecule comprises a compound comprising a nucleic acid 8 to 50 nucleotides in length, wherein said compound specifically hybridizes with a polynucleotide encoding Prox-1, or 25 hybridizes to the complement of the polynucleotide, and inhibits the expression of Prox-1 when introduced into a cell that expresses Prox-1.

69. The use of claim 68, wherein the compound is an antisense oligonucleotide.

70. The use of claim 69, wherein the antisense oligonucleotide has
5 a sequence complementary to a fragment of SEQ ID NO: 1.

71. The use of claim 70, wherein the fragment of SEQ ID NO: 1
comprises a promoter or other control region, an exon, an intron, or an exon-intron
boundary.

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72. The use of claim 70, wherein the fragment of SEQ ID NO: 1
comprises an exon-intron splice junction.

73. The use of claim 70, wherein the fragment of SEQ ID NO: 1
15 comprises a region within 50-200 bases of an exon-intron splice junction.

74. The method or use according to any one of claims 16-21,
wherein the molecule comprises an inhibitor of DNA methyltransferases, thereby
inhibiting Prox-1 expression.

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75. The method or use according to claim 74, wherein the inhibitor
of DNA methyltransferases is 5-aza-2'-deoxycytidine.

76. The method according to any one of claims 22-31, further
25 comprising administering to the subject an inhibitor of DNA methyltransferases.

77. The use according to any one of claims 22-30, wherein the
medicament further includes an inhibitor of DNA methyltransferases.

78. The method or use of claim 76 or 77, wherein the inhibitor of DNA methyltransferases is 5-aza-2'-deoxycytidine.

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